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CELL-SIZE DISTRIBUTIONS OF ZOOXANTHELLAE IN CULTURE AND SYMBIOSIS

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Abstract

Zooxanthellae maintained in batch culture at 27°C with a 12 h:12 h light/dark cycle exhibited different cell-size distributions and morphology with growth phase. Mean cell diameter of cultured zooxanthellae isolated from *Zoanthus sociatus* increased with time of culture and growth phase from 6 μm (day 3) to 12.5 μm (day 11, late log-phase growth). Maximum specific growth rates (μ_{max} $\log_e$ units) ranged from 0.3 to 0.4 day^{-1}. Within three hours after the onset of the light period on the third day of culture (early log-phase growth), large numbers of motile cells appeared. Subsequently, the dominant cell type was non-motile, vegetative, and possessed an accumulation body which increased in size with culture time. Zooxanthellae freshly isolated from a variety of hosts in their natural environment exhibited different cell-size distributions on an inter- and intra-host basis. Based on the laboratory results with cultured zooxanthellae, varying size distributions in fresh isolates of zooxanthellae from natural hosts may be explained by growth-related factors.

Introduction

Zooxanthellae (endozoic dinoflagellates) are the most prevalent algal symbionts of coral reef invertebrates (Taylor, 1974). Their role in host nutrition (Muscatine, 1980; D'Elia *et al.*, 1983) and the ecology of reef communities (Muscatine and Porter, 1977) is well studied. One encounters considerable debate in the literature about the systematics, distribution, and number of species and strains of zooxanthellae in existence: little consensus has yet emerged.

Recent work with cultured zooxanthellae indicates there are a variety of genetically different species (Blank and Trench, 1985) or strains that differ in morphology and cell diameter (Schoenberg and Trench, 1980a), mechanisms of photoadaptation (Chang *et al.*, 1983), patterns of motility (Fitt *et al.*, 1981; Lerch and Cook, 1984), and host selectivity (Schoenberg and Trench, 1980b).

Zooxanthellae in laboratory culture have a complex life history that alternates between motile gymnodinoid and non-motile coccoid stages exhibiting different cell size under certain conditions (Freudenthal, 1962; Trench, 1981). However, the relationship between cell-size distribution and morphology is poorly understood for zooxanthellae in culture, and there has been little attempt to relate the phase of growth of zooxanthellae to their morphological or physiological characteristics. In this paper, we characterize changes in cell size and morphology with growth phase for zooxanthellae in culture and examine natural variations in cell-size distributions for zooxanthellae freshly isolated from a variety of reef invertebrates.

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Materials and Methods

Batch cultures

Gymnodinium microadriaticum isolated in Discovery Bay, Jamaica, from the Caribbean zoanthid *Zoanthus sociatus* was maintained in 100-ml batch cultures at 27°C, 12:12 h L:D period, 45 μEin $m^{-2}s^{-1}$ using the methods of Domotor and D'Elia (1984). Cultures were less than one year of age from the original isolation. To facilitate accurate determination of zooxanthellae division rate, cell size, and size distributions, cells in a 100-ml batch culture were scraped from flask surfaces with a rubber policeman and passed through 20-μm mesh Nitex screening by gentle agitation. This procedure separated clumped cells and produced a uniform suspension of algae but caused no damage to them; cells were intact and viable as verified by examination with bright-field microscopy.

Division and growth-rate determinations

Division and growth rates were determined in six trials (individual cultures) of *Gymnodinium microadriaticum.* Division rates were determined by monitoring increase with time in cell numbers using an Electrozone Celloscope particle counter (Particle Data, Inc., Elmhurst, Illinois) equipped with a 120-μm aperture tube. Growth rate was determined by monitoring changes in *in vivo* chlorophyll *a* fluorescence with time using a Turner Designs model 10 fluorometer (Mountain View, California) with CS-5-60 excitation and Wratten CS-2-64 emission filters.

Cell size distribution and morphology in cultured zooxanthellae

Cell sizes and their distributions were monitored at 1300 local time every 1–3 days over a 25-day period with the Electrozone computerized particle data system interfaced to a Digital PDP 8 computer. On several occasions bright-field microscopy was used to verify cell-size data obtained from the particle data system. Cells were examined for morphological changes using bright-field microscopy in conjunction with cell-size analysis.

Cell-size distribution in freshly isolated zooxanthellae

Zooxanthellae were isolated from a variety of reef corals, tridacnid clams, and zoanthids as described in D'Elia *et al.* (1983). Ten ml of freshly isolated zooxanthellae suspension were pipetted into plastic scintillation vials and fixed with an I_2-KI fixative (Utermöhl, 1958) for future analysis. Cell-size-distribution analysis was as described above for cultured zooxanthellae.

Control experiments

To determine if preservation with I_2-KI altered cell size, zooxanthellae isolated from several of the invertebrates sampled were examined using bright-field microscopy before and after addition of I_2-KI. Cultured zooxanthellae were examined using the particle data system before and after addition of I_2-KI. Comparisons of cell size before and after preservation indicated that cell sizes and size distribution were unaffected by the I_2-KI fixative. Examination of freshly isolated zooxanthellae with bright-field microscopy indicated that isolations were free of animal tissue fragments and bacteria that may have biased cell-size distribution determinations.

Results

Division rates of cultured zooxanthellae

Maximum specific division rates of cultured zooxanthellae were determined on six different occasions using cell-count and *in vivo*-fluorescence techniques. Mean maximum division rates (μ_m in $\log_e$ units) from the two techniques were not significantly different (*t*-test, n = 6, $P < 0.05$), yielding means (±2 S.E.) of 0.35 ± 0.08 day^{-1} and 0.39 ± 0.18 day^{-1}, respectively.

Growth characteristics of cultured zooxanthellae

In culture, zooxanthellae isolated from *Zoanthus sociatus* were predominantly non-motile coccoid cells that clumped together and adhered to culture vessel surfaces, a characteristic noted by other workers who have maintained zooxanthellae in the laboratory (Freudenthal, 1962; Loeblich and Sherley, 1979). Gymnodinoid stages were observed predominantly in 3-day-old cultures corresponding to early log phase, with motility most noticeable about 3 h after the onset of the light period and lasting for approximately 0.5 h. These motility patterns were consistently observed for every subculturing of zooxanthellae, however, we emphasize that we made no systematic investigation of this phenomenon and such observations should be considered preliminary. Motile cells were positively phototactic and attached themselves to flask walls nearest to the light source.

Changes in cell size and morphology for cultured zooxanthellae

Figure 1 shows the size distribution of *Gymnodinium microadriaticum* with culture time and illustrates cell morphological types corresponding to peak cell sizes. There was a progressive increase in peak cell diameter from 6 μm (day 3) to 12.5 μm (day 11) with the greatest increase in the frequency of large cells occurring toward the end of log-growth phase. Cell diameter remained constant during days 11–25 of culture.

Cell size distribution from freshly isolated zooxanthellae

Primary and secondary peak cell sizes for zooxanthellae immediately isolated from invertebrate hosts sampled at different geographical locations are presented in Table I. The majority of the hosts sampled harbored two distinctly different size classes of zooxanthellae: a primary peak cell size of 8–14 μm and a secondary peak cell size of 5–7 μm (with the exception of *Montastrea* which had a primary peak cell size of 5 μm and a secondary peak cell size of 10 μm). Peak cell sizes (Table I) and actual size distributions (Fig. 2) of zooxanthellae isolated from the hosts sampled show that distinct differences in cell-size distributions exist on an inter- and intra-host basis. Replicate size distributions of zooxanthellae isolated from a coral (*Seriatopora*), a bivalve (*Tridacna*), and a zoanthid (*Zoanthus*) are variable in size range and frequency (Fig. 2).

Discussion

Variations in cell morphology (*i.e.*, between life-cycle stages) are well known for zooxanthellae in culture, but changes in cell sizes and size distributions in relation to growth phase of a given stage have not been reported previously. Changes in cell diameter (from 6 μm to 12.5 μm) and cell-size distribution were observed particularly at the end of log growth with accompanying changes in cell morphology. We do not

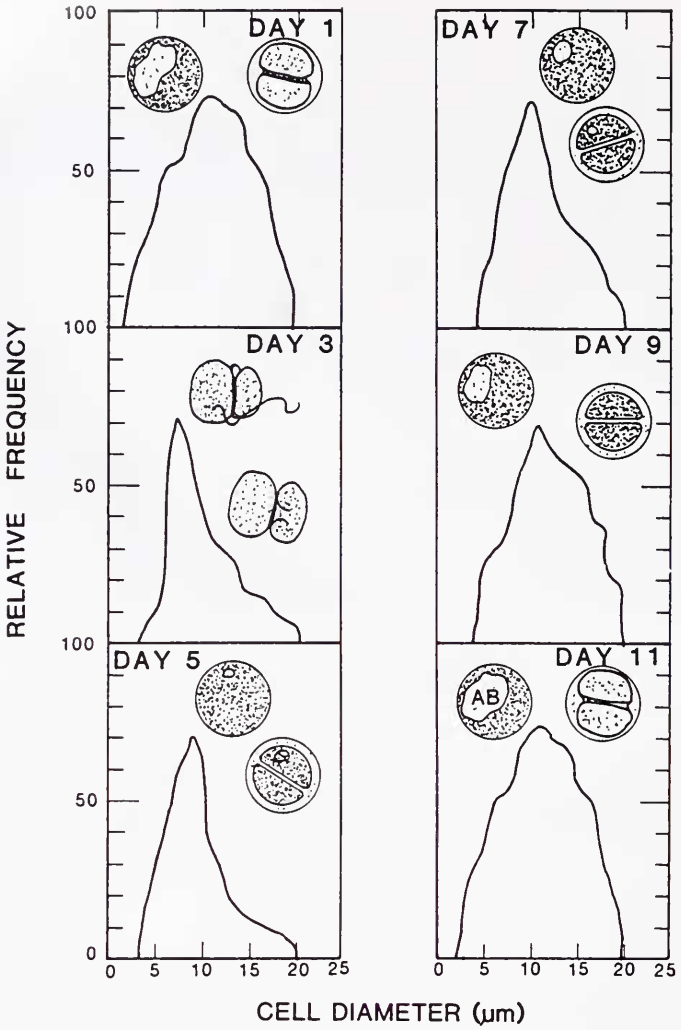

FIGURE 1. Size distribution of *Gymnodinium microadriaticum* with culture time. Observed cell morphological types corresponding to peak cell sizes are illustrated. Changes in cell morphology with culture time were: Day 1 = day of inoculation; aging vegetative cells and zoosporangia. Day 3 = gymnodinoid cells and dumbbell-shaped non-motile cells after dropping their flagella and attaching to solid surface; Days 5–9 = vegetative cells dividing to produce two daughter cells and vegetative cells aging with accumulation body enlarging as growth proceeds; Days 11–25 = aging vegetative cells with large accumulation bodies; some zoosporangia (AB = accumulation body). Two other cultures of zooxanthellae gave similar size distributions with culture time. There was no change in cell-size distribution during days 11–25.

know if cell-size and morphology for zooxanthellae in intact symbioses also vary with division rate, but we believe the possibility should be investigated.

Natural variations of cell-size distributions for zooxanthellae isolated from reef invertebrates occurred in this study on an inter- and intra-host basis. This could indicate (1) the presence of host-specific strains or species of zooxanthellae with different size

TABLE I

Primary and secondary peak cell sizes (based on percent of total cell number) of zooxanthellae isolated from a variety of reef invertebrates collected from a variety of geographical locations

Location of collection	Invertebrate host	Primary peak cell size (μm)	Secondary peak cell size (μm)
Cayos Arcas, Mexico	*Montastrea* sp.	5.2	10.2
Kaneohe Bay, Hawaii	*Zoanthus* sp.	8.9	
		8.5	
		7.5	
		$\bar{x} \pm$ S.E. = 8.30 $\pm$ 0.72	
Kaneohe Bay	*Pocillopora* sp.	13.8	7.0
Swains Reefs, Great Barrier Reef, Australia	*Seriatopora* sp.	8.9	5.5
		9.7	
		9.1	
		9.7	
		$\bar{x} \pm$ S.E. = 9.35 $\pm$ 0.41	
Swains Reefs	*Fungia* sp.	7.7	
Swains Reefs	*Acropora* sp.	11.5	5.2
Swains Reefs	*Tridacna* sp.	9.7	5.0
		12.3	
		10.2	
		$\bar{x} \pm$ S.E. = 10.73 $\pm$ 1.38	

and distribution characteristics, (2) variability of zooxanthellae growth and morphology in these symbioses, or (3) both. The presence of two distinct size classes of zooxanthellae in the majority of the invertebrates sampled is consistent with both possibilities, but at present we can only speculate that the high frequency of large cells observed in freshly isolated zooxanthellae correlates with late-log or stationary-phase growth.

Similarity between growth rates of cultured zooxanthellae calculated from cell counts and growth rates as determined by *in vivo* fluorescence measurements indicates that the two methods give comparable results. Maximum specific growth rates compare to those reported by other workers (*c.f.,* Loeblich and Sherley, 1979; Chang *et al.,* 1983).

Patterns of motility were similar to those observed by other workers (Fitt *et al.,* 1981; Lerch and Cook, 1984). Although motile stages of zooxanthellae may have been present throughout log phase, they were most noticeable on day three of early log-phase growth. Given the presence of large numbers of zoospores in stationary phase and the peak occurrence of motility in early log phase of culture, some algal or environmental factor apparently triggers the onset of motility.

The regulation of cell size, morphology, and growth/division rate of zooxanthellae in intact symbiosis is not well understood. Several possible factors or combinations of factors exist: (1) cell size, morphology, and growth rate are regulated by the zooxanthellae themselves, (2) these characteristics are regulated in some fashion by a host factor or nutrition, and (3) they are regulated by the environmental conditions in which the symbioses occur (reviewed by Cook, 1984).

If what we observed for cultured zooxanthellae in the laboratory applies to zooxanthellae in their hosts, varying morphological stages and cell sizes may correlate with varying growth rates, and factors related to growth and nutrition of the algae may be implicated. Future efforts seem warranted to determine the mechanisms that regulate zooxanthellae growth rate, cell size, and morphology by maintaining zooxanthellae

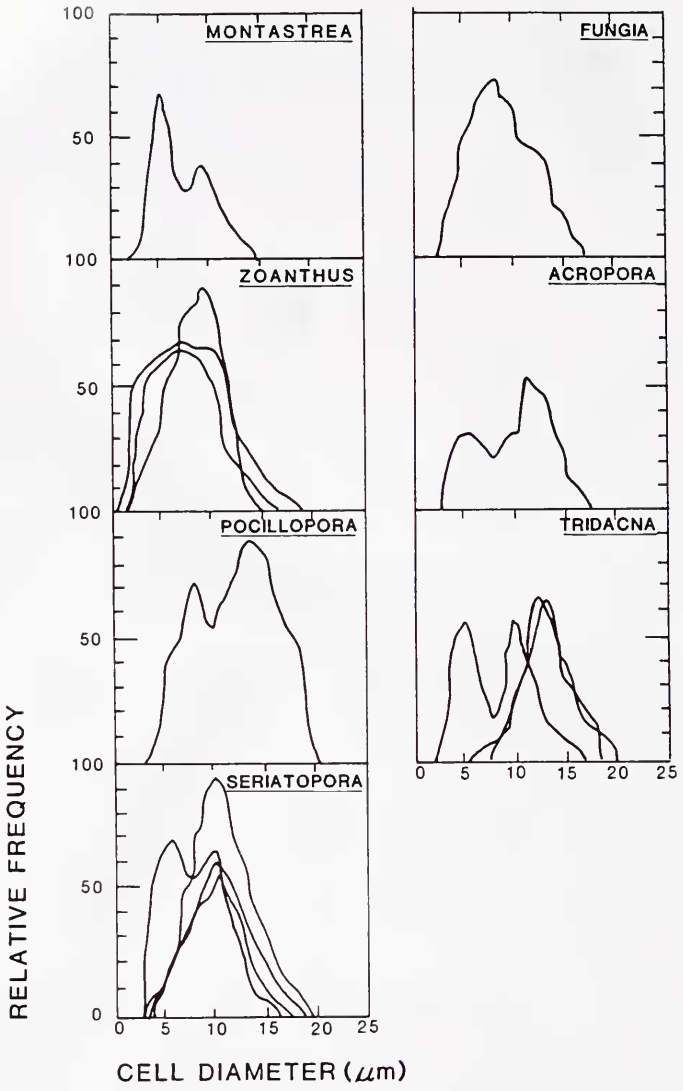

FIGURE 2. Cell-size distributions of zooxanthellae isolated from a variety of reef invertebrates collected from different locations. Replicate algal size distributions are presented for several of the hosts sampled.

under a variety of nutrient conditions (nutrient concentrations, rates of supply and sources) and light regimes (light quantity, quality, and photoperiod). Inasmuch as most studies employing cultured algae are done with clones maintained in the laboratory for months to years with progressive subculturing, an important question that needs addressing is whether zooxanthellae change nutritionally, physiologically, or morphologically with culture time from original isolation from the intact association through subsequent subculturing.

Clearly, more work with cultured zooxanthellae maintained under defined environmental conditions will help provide a better understanding of the mechanisms

that regulate zooxanthellae cell size, morphology, and growth rate. The information obtained in such studies will be particularly useful if related to zooxanthellae in symbiosis.

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LITERATURE CITED

BLANK, R. J., AND R. K. TRENCH. 1985. Speciation and symbiotic dinoflagellates. *Science* **229:** 656–658.

CHANG, S. S., B. B. PREZELIN, AND R. K. TRENCH. 1983. Mechanisms of photoadaptation in three strains of the symbiotic dinoflagellate *Symbiodinium microadriaticum*. *Mar. Biol.* **76:** 219–229.

COOK, C. B. 1984. Equilibrium populations and long-term stability of mutualistic algae and invertebrate hosts. In *The Biology of Mutualism: Ecology and Evolution,* D. Boucher, ed. Croon-Helm, Amsterdam. In press.

D'ELIA, C. F., S. L. DOMOTOR, AND K. L. WEBB. 1983. Nutrient uptake kinetics of freshly isolated zooxanthellae. *Mar. Biol.* **75:** 157–167.

DOMOTOR, S. L., AND C. F. D'ELIA. 1984. Nutrient uptake kinetics and growth of zooxanthellae maintained in laboratory culture. *Mar. Biol.* **80:** 93–101.

FITT, W. K., S. S. CHANG, AND R. K. TRENCH. 1981. Motility patterns of different strains of the symbiotic dinoflagellate *Symbiodinium* (=Gymnodinium) *microadriaticum* (Freudenthal) in culture. *Bull. Mar. Sci.* **31:** 436–443.

FREUDENTHAL, H. 1962. *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxanthellae; taxonomy, life cycle, and morphology. *J. Protozool.* **9:** 45–52.

LERCH, K. A., AND C. B. COOK. 1984. Some effects of photoperiod on the motility of rhythm of cultured zooxanthellae. *Bull. Mar. Sci.* **34:** 477–483.

LOEBLICH, A. R., AND J. L. SHERLEY. 1979. Observations on the theca of the motile phase of free-living and symbiotic isolates of *Zooxanthellae microadriatica* (Freudethal) comb. nov. *J. Mar. Biol. Assoc. U. K.* **59:** 195–205.

MUSCATINE, L. 1980. Uptake, retention, and release of dissolved inorganic nutrients by marine alga-invertebrate associations. Pp. 229–244 in *Cellular Interactions in Symbiosis and Parasitism,* C. B. Cook *et al.,* eds. Ohio State University Press, Columbus.

MUSCATINE, L., AND J. W. PORTER. 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *BioScience* **27:** 454–459.

SCHOENBERG, D. A., AND R. K. TRENCH. 1980a. Genetic variation in *Symbiodinium (=Gynodinium) microadriaticum* Freudenthal and specificity in its symbiosis with marine invertebrates. II. Morphological variation in *S. microadriaticum. Proc. R. Soc. Lond. Ser. B* **207:** 429–444.

SCHOENBERG, D. A., AND R. K. TRENCH. 1980b. Genetic variation in *Symbiodinium (= Gymnodinium) microadriaticum* (Freudenthal), and specificity in its symbiosis with marine invertebrates. III. Specificity and infectivity of *Symbiodinium microadriaticum. Proc. R. Soc. Lond. Ser. B.* **207:** 445–460.

TAYLOR, D. L. 1974. Symbiotic marine algae: taxonomy and biological fitness. Pp. 245–252 in *Symbiosis in the Sea,* B. Vernberg, ed. University of South Carolina Press, Columbia.

TRENCH, R. K. 1981. Cellular and molecular interactions in symbioses between dinoflagelates and marine invertebrates. *Pure Appl. Chem.* **53:** 819–835.

UTERMÖHL, H. 1958. Zur Vervollkommung der Quantitativen Phytoplankton-Methodik. *Mitt. Int. Verein. Theor. Angew. Limnol.* **9:** 1–38.